Septins and a formin have distinct functions in anaphase chiral cortical rotation in the C. elegans zygote.

Adhham Zaatri, Jenna Perry, and Amy Maddox

Corresponding author(s): Amy Maddox, UNC-Chapel Hill

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RE: Manuscript #E20-09-0576
TITLE: "Septins and a formin have distinct functions in anaphase chiral cortical rotation in the C. elegans zygote."

Dear Amy, your manuscript "Septins and a formin have distinct functions in anaphase chiral cortical rotation in the C. elegans zygote" has now been reviewed by two referees. While the two differ slightly in terms of their overall enthusiasm, it is clear that both of them consider this study interesting and of high quality. I am therefore pleased to tell you that Molecular Biology of the Cell would be happy to accept this manuscript if you address the reviewer comments with the appropriate revisions to the text.

Many thanks for submitting this work to MBoC.

Bill Bement

Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Maddox,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

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Authors of Articles and Brief Communications whose manuscripts have returned for minor revision ("revise only") are encouraged to create a short video abstract to accompany their article when it is published. These video abstracts, known as Science Sketches, are up to 2 minutes long and will be published on YouTube and then embedded in the article abstract. Science Sketch Editors on the MBoC Editorial Board will provide guidance as you prepare your video. Information about how to prepare and submit a video abstract is available at www.molbiolcell.org/science-sketches. Please contact mboc@ascb.org if you are interested in creating a Science Sketch.

Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

Zaatri et al use C. elegans embryos to study actomyosin driven protein-to-cell scale chirality by septins and the diaphanous formin homolog CYK1 during cell division. Septins are required for anaphase associated rotations, whereas, this particular formin contributes to overall embryo chirality but is not specific to anaphase associated rotations. Mutants lacking both septin and formin resulted in reversed embryo chirality. Overall this work is a really nice mix of beautifully quantified data, physics, and cell biology. This work is written in a very approachable manner and this reviewer supports its publication with minor edits / clarifications. The following are minor comments:

• The authors mention that there are 2 septin genes in worms, but it takes a while for this point to be clear. I would state this earlier, possibly in the introduction, just so the reader has more instant gratification that both/all forms are being tested in this work.
• The methods section is very detailed and clear. The A-P abbreviation on the top of page 8 should be spelled out for consistency because this was the only place it was abbreviated in the text.
• The 0.35 µm/s velocity of cortical rotation is intriguing but it wasn't really clear what this meant for the "big picture". Does chirality speed matter? Is this speed dictated by actin polymerization? Is this velocity similar to predicted rates of actin or formin-based actin polymerization in worms (or other systems)? If so, it might be worth mentioning this for the uninitiated. Additionally, perhaps the authors could comment on what happens to this velocity when actin polymerization in worms is perturbed with drug treatments like LatA or JASP. Or even treatments that would unlink microtubules from this process.
• It is really compelling that the two septin mutants phenocopy each other (and the siRNA). Is a septin-CYK1 double mutant or septin-septin-cyk1 triple mutant viable?
• While this is beyond the scope of this work, have the authors considered "rescuing" the formin with characterized point mutants in the FH2 domain that should be able to separate the actin polymerization from other possible functions in cells?
• The discussion was enjoyable, stimulating, and clear concerning the role of how chirality and rotation translates/manifests in mammalian and worm systems. It left this reviewer really curious to read the author's perspective on a couple of papers utilizing other Diaphanous formins from different in vitro systems, particularly: Mizuno et al 2018, Jejou et al 2013, and Zimmermann et al 2017. It may be valuable to readers to make further connections between the septin-formin interaction quantified here, across model systems.

Reviewer #2 (Remarks to the Author):

The manuscript titled "Septins and a formin have distinct functions in anaphase chiral cortical rotation in the C. elegans zygote" by Zaatri et al addresses the very interesting question of molecular mechanisms determining chirality during anaphase of cell division in the C.elegans zygote. The authors show that Septin mutants lead to a block in cortical rotation. They further analyse the distribution Cyk-1 in Septin mutants and find that it is not present at the posterior cortex. Cyk-1 mutants show reversal in rotation at the cortex. Septin and Cyk-1 mutants show a convincing loss and perturbation of chirality. The double mutants of Septin and Cyk-1 show that Septins are involved in establishing chirality and Cyk-1 is determining the directionality of chirality. The data are interesting and the phenotypic characterisation is done well. However, it would be nice to add a further mechanistic interpretation of the link between Septins and Cyk-1 based on the comments below in order to address the role that Septins and Cyk-1 play during chirality.

Septin distribution in the zygote during the chiral rotation stages is not addressed in the manuscript from literature as well as in experiments. Is it possible for the authors to document or discuss the analysis with Septin distribution during the chiral rotation events? Is it uniformly present across the embryo and is there a flow/directed recruitment of Septins towards the cleavage furrow in the same axis as the chirality occurs?

Cyk-1 distribution is abrogated on depletion of Septins. Does the Septin distribution in turn depend upon Cyk-1? How do Septins affect the polarised distribution of Cyk-1?

Cyk-1 depletion unlike Septin depletion shows reversed handedness, are there examples of other mutants that do the same? Do these mutants all belong to a class of actin bundling proteins?

Will an analysis of the actin cytoskeleton dynamics or distribution at the cortex during rotation in Septin and Cyk-1 mutant embryos give more direct information for directionality of rotation?

Each plot for circumferential movement and antero-posterior flow shows average data in a dark line and individual plots in differently colored weaker lines in the background. Statistics on each of the average plots of the mutants as compared to the controls would complete the analysis from the plot for assessment of the data.

Representative movies for double mutants should be added as supplemental data.

The labels in the schematic in Figure 5 could be placed outside the drawing in order to allow for better visibility for the

mechanism documented in the schematic.

Dear Bill, Eric, and colleagues,

We were grateful to receive the referees' thoughtful and thorough critiques, and to strengthen our manuscript according to their recommendations. Please find point-by-point responses below, noting where in the manuscript file changes have been made.

We apologize for the delay and thank you for the extension.

We look forward to hearing from you, and will appreciate your attention to our revision, as an editorial decision could be crucial for a post-submission update to accompany recent funding proposals.

Thank you for your support and continued consideration.

Sincerely,
Amy

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To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL):
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• The authors mention that there are 2 septin genes in worms, but it takes a while for this point to be clear. I would state this earlier, possibly in the introduction, just so the reader has more instant gratification that both/all forms are being tested in this work.

>We apologize for this oversight and appreciate the opportunity to improve the manuscript according to this thoughtful suggestion (please see page 4 in the manuscript text).

• The methods section is very detailed and clear. The A-P abbreviation on the top of page 8 should be spelled out for consistency because this was the only place it was abbreviated in the text.

>This has been done. Thank you! (see page 8)

• The 0.35 µm/s velocity of cortical rotation is intriguing but it wasn't really clear what this meant for the "big picture". Does chirality speed matter?

>It is difficult to say whether the speed of chiral rotation matters, since perturbations that change it also change contractility in general and therefore compromise cell division and animal development, but not necessarily via their effect on chiral cortical rotation. We think that cortical rotation provides a readout of the torsional stress in the actomyosin network, and thus the

abundance of polymer and crosslinking sites. Our hypothesis has been clarified in the Discussion.

Is this speed dictated by actin polymerization?

>We cannot answer this, because it has not been examined in a condition in which the speed of actin polymerization has been modified, just in cases with depressed abundance of polymerization (profilin depletion eliminates rotation (Schonegg, Hyman & Wood, *Genesis* 2014) and CYK-1 formin depletion causes a range of defects (our work)). That said, rotation is slower than the rate of F-actin polymerization in the *C. elegans* zygote in vivo, as estimated by tracking single molecules of fluorescently-tagged CYK-1 (Li & Munro *BioRxiv* https://doi.org/10.1101/2020.04.13.039586).

Is this velocity similar to predicted rates of actin or formin-based actin polymerization in worms (or other systems)? If so, it might be worth mentioning this for the uninitiated.

>The speed of cortical rotation is 2.5-9 x faster than that of NMMIIs in vitro (this range reflects the difference in velocity between NMMIIA (faster, in methylcellulose) and NMMIIB (slower, measured in buffer); Melli, Sellers *et al, eLife* 2018)). Cortical rotation speed is slower than CYK-1 and related formins' single molecule movement in vivo (1.5-2.0 μm/s; (Li & Munro *BioRxiv* https://doi.org/10.1101/2020.04.13.039586, Higashida, Watanabe *et al., Science* 2004). Since rotation is faster than NMMII movement, we think it results from a relief of torsional stress within the whole-cell cortical network, by cytokinetic ring contractility. These speed comparisons, and our hypothesis about the relief of stress, have been added to our Discussion (see pages 15-16).

Additionally, perhaps the authors could comment on what happens to this velocity when actin polymerization in worms is perturbed with drug treatments like LatA or JASP. Or even treatments that would unlink microtubules from this process.

>Chiral rotation has not been examined following treatment with LatA or jasplakinolide, but this would be very interesting to try in the future. Rotation has also not been examined following depletion of an F-actin-microtubule crosslinker such as VAB-10 (the *C. elegans* homologue of *Drosophila* Slingshot).

Cortical rotation has been examined in conditions where microtubules are generally much shorter and less likely to reach from centrosomes to the cortex (treatment with nocodazole or depletion of tubulin, Aurora A, and other components of the centrosome) and following two perturbations that should reduce microtubule-cortical interactions (depletion of dynein heavy chain and LIN-5 (a component of the G-alpha, dynein microtubule anchoring complex) (Schonegg, Hyman & Wood, *Genesis* 2014). These findings and the general requirement for the actomyosin cytoskeleton for *C. elegans* zygote cytokinetic rotation are briefly summarized in the Introduction (middle of page 3).

• It is really compelling that the two septin mutants phenocopy each other (and the siRNA). Is a septin-CYK1 double mutant or septin-septin-cyk1 triple mutant viable?

>CYK-1 is an essential protein and animals lacking CYK-1 function are not viable (likely due to its essential role in cytokinesis). Partial CYK-1 depletion (via intermediate timepoints of *cyk-1(RNAi)*) will lead to embryonic lethality with incomplete penetrance (with lethality itself being a binary score per embryo). It would be therefore possible to test, in the future, whether the penetrance of "partial" lethality following partial loss of CYK-1 function is exacerbated by loss of septin function.

• While this is beyond the scope of this work, have the authors considered "rescuing" the formin with characterized point mutants in the FH2 domain that should be able to separate the actin polymerization from other possible functions in cells?

>It is interesting to consider how mutations in the FH2 domain that perturb F-actin bundling and/or F-actin-microtubule association (Ishizaki, Narumiya *et al., Nat Cell Biol* 2001) would affect cortical rotation. We predict they could phenocopy septin loss of function by decreasing the connectivity within the actomyosin cytoskeleton in a molecularly distinct but functionally similar way as septin loss of function.

• The discussion was enjoyable, stimulating, and clear concerning the role of how chirality and rotation translates/manifests in mammalian and worm systems. It left this reviewer really curious to read the author's perspective on a couple of papers utilizing other Diaphanous formins from different in vitro systems, particularly: Mizuno et al 2018, Jejou et al 2013, and Zimmermann et al 2017. It may be valuable to readers to make further connections between the septin-formin interaction quantified here, across model systems.

>We thank the referee for bringing these publications to our attention. We should have referenced them before, and now we do. We now say in our Discussion, “Without membrane anchoring, the formin would freely rotate around the associated F-actin over the course of polymerization (Jegou et al, 2013, Zimmerman et al, 2017, Mizuno et al, 2018), and torsional stress would not accumulate.” We do not go further, in our Discussion, than mentioning this torsional stress, because the three noted papers suggest that F-actin may be less stable in septin depleted embryos, if it is under less torsional stress and therefore more susceptible to severing by cofilin, or that the formin would more readily polymerize since there would be less resistive force on the formin that would otherwise limit its activity. These predictions are difficult to distinguish but it seems safe to say that without anchoring, the freedom of movement of the formin during F-actin assembly reduces the accumulation of torsional stress. We thank the referee for suggesting the addition of these important references.

Reviewer #2 (Remarks to the Author):

The manuscript titled "Septins and a formin have distinct functions in anaphase chiral cortical rotation in the C. elegans zygote" by Zaatri et al addresses the very interesting question of molecular mechanisms determining chirality during anaphase of cell division in the C. elegans zygote. The authors show that Septin mutants lead to a block in cortical rotation. They further analyse the distribution Cyk-1 in Septin mutants and find that it is not present at the posterior cortex. Cyk-1 mutants show reversal in rotation at the cortex. Septin and Cyk-1 mutants show a convincing loss and perturbation of chirality. The double mutants of Septin and Cyk-1 show that Septins are involved in establishing chirality and Cyk-1 is determining the directionality of chirality. The data are interesting and the phenotypic characterisation is done well. However, it would be nice to add a further mechanistic interpretation of the link between Septins and Cyk-1 based on the comments below in order to address the role that Septins and Cyk-1 play during chirality.

<u>Septin distribution</u> in the zygote during the chiral rotation stages is not addressed in the manuscript from literature as well as in experiments. Is it possible for the authors to document or discuss the analysis with Septin distribution during the chiral rotation events? Is it uniformly present across the embryo and is there a flow/directed recruitment of Septins towards the cleavage furrow in the same axis as the chirality occurs?

> We regret neglecting to discuss what is known about septin distribution: it is enriched in the zygote anterior and the cytokinetic ring (Jordan, Canman et al, JCB 2016). We now reference this in the text (please see page 4, near top). In response to the referee's request, we have added our own observations of septin distribution. As previously shown, septin is enriched in the anterior (Fig. 3 supplement, panel A).

Since the entire cortex, and even the spindle, rotates, cortical septin is expected to rotate, and we can say anecdotally that it does. This former point about structures from the cortex to deep in the cytoplasm all rotating has been added to the Introduction.

Cyk-1 distribution is abrogated on depletion of Septins. Does the Septin distribution in turn depend upon Cyk-1? …

> We appreciate the suggestion and are glad to add this important experiment to the manuscript. We now report that septin distribution is not perturbed by depletion of CYK-1. Since this result does not impact our conclusions, we have included these data as a supplemental figure (Figure 3 supplement, panel A).

How do Septins affect the polarised distribution of Cyk-1?

>As the referee states above, the effect of septin depletion on the polarised distribution of CYK-1 was originally presented (Figure 3).

Cyk-1 depletion unlike Septin depletion shows reversed handedness, are there examples of other mutants that do the same? Do these mutants all belong to a class of actin bundling proteins?

> Reversed handedness of rotation has been observed following perturbation of anterior-posterior polarity by depletion of PAR-2,-3, or -6 or CDC-42 (handedness was reversed in 50%, 16%, 18%, and 25% of cells, respectively; Schonegg *et al.* 2014). Perturbation of anterior-posterior polarity is likely to alter the polarization of CYK-1, which we predict influences rotation chirality. This mechanistic connection has been added to the Discussion and we thank the referee for triggering this line of thought.

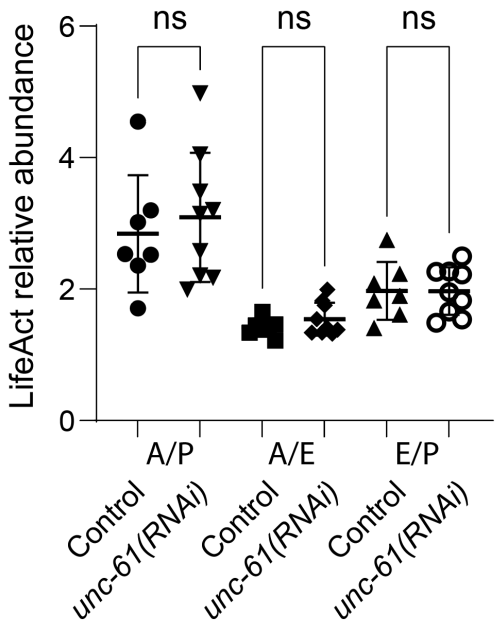
A low incidence of handedness-reversal was also observed in zygotes depleted of centralspindlin proteins (ZEN-4 (20%) and CYK-4 (8%); Schonegg *et al.* 2014). Depletion of Casein kinase 1- gamma and members of the Wnt pathway (including Wnt MOM-2) also caused revered-handedness (Singh, Pohl *et al. J Cell Science* 2019). Though some or all of these upstream regulators may influence anterior-posterior polarity, or F-actin bundling proteins as the referee mentions, no direct mechanistic connection is clear from these published results.

Will an analysis of the actin cytoskeleton dynamics or distribution at the cortex during rotation in Septin and Cyk-1 mutant embryos give more direct information for directionality of rotation?

>We greatly appreciate the suggestion to test some of the hypotheses in our Discussion, and the opportunity to strengthen our manuscript via the addition of these results. We examined the polarization of the actomyosin cortical cytoskeleton. Its known anterior enrichment supported the idea that asymmetric/polarized torsional stress can accumulate. We hypothesized that the polarization of the cortex could affect the chirality of the torsional stress, and therefore cortical rotation, with respect to the anterior-posterior axis. We found that CYK-1 depletion diminishes to anterior enrichment of F-actin, very significantly so in cells exhibiting cortical rotation with opposite handedness (Fig. 4I).

We also found that, in agreement with previous observations that septin loss of function does not affect anterior-posterior polarity, F-actin asymmetry/polarization was not affected by depletion of septins. LifeAct fluorescence intensity was measured in a rectangular region occupying much of the anterior (A), equator (E), or posterior (P) cortex in each of three cortical

focal planes, and averaged the 3 measurements per region. Intensity values per region per cell were corrected by subtracting that of a region outside the cell. The lack of a statistically significant difference ("ns") between control and UNC-59 depleted cells was demonstrated using Prism software. Since these results do not advance the manuscript, we only include them here.



Each plot for circumferential movement and antero-posterior flow shows average data in a dark line and individual plots in differently colored weaker lines in the background. Statistics on each of the average plots of the mutants as compared to the controls would complete the analysis from the plot for assessment of the data.

>We have added to the supplements to Figures 2 and 4 plots of circumferential and posterior-directed velocity means +/- one standard deviation. We are grateful for this suggestion which we feel strengthens our data presentation. (see Figure 2 supplement and Figure 4 supplement)

Representative movies for double mutants should be added as supplemental data.

>This has been done. Thank you!

The labels in the schematic in Figure 5 could be placed outside the drawing in order to allow for better visibility for the mechanism documented in the schematic.

>This has been done. Thank you!

RE: Manuscript #E20-09-0576R
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Dear Amy,

Many thanks for handling the revisions thoroughly. I am pleased to tell you that your paper is now suitable for publication by Molecular Biology of the Cell. My thanks as well for your decision to submit this work to MBoC.

Bill

Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Maddox:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

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We are pleased that you chose to publish your work in MBoC.

Sincerely,

Eric Baker
Journal Production Manager
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